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# Plasma Renin Activity and Aldosterone: Correlations with Moderate Hypohydration

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#### **Abstract**

Adult male test subjects (n=16) were assigned to one of three clothing configurations (Army fatigues, fatigues with chemical protective garments, and fatigues with protective garments plus protective masks) prior to exercise (level treadmill, 1.11 m/s, 50 min/h, 6 h) in a moderate (WBGT = 23°C) environment with ad lib water consumption. Antecubital blood samples were taken prior to the start of and subsequent to the completion of exercise and analyzed for fluid-electrolyte regulatory hormones. During all trials with chemical protective garments, plasma renin activity (PRA) and aldosterone levels (PA) were significantly (p<0.05) elevated following the exercise protocol while neither was affected during exercise in fatigues only. Individual hypohydration levels during all trials ranged from low (0.84%) to moderate (4.04%). Levels of PRA were closely correlated (r=0.635, t=4.35, p<0.001) with hypohydration as measured by percentage of body weight lost during the 6 h trial. Likewise, PA was also correlated (r=0.47, t=2.81, p<0.01) with body weight loss. We concluded from this study that PRA and PA responses were exacerbated in moderate environments by the additional heat stress of impermeable garments. Further, the logistical difficulty inherent in delivering fluid through the chemical protective mask reduced voluntary consumption, increased hypohydration, and elicited the greatest elevations in PRA and PA. Finally, even at these modest levels of hypohydration, the intensity of the PRA and PA responses were correlated with hypohydration level.

KEY WORDS: exercise, hormonal responses, fluid-electrolyte regulation,
protective clothing

## Introduction

In his recent book Rowell (30) notes three generalized mechanisms by which the biosynthesis of renin may be stimulated: increased renal sympathetic activity which is sensitive to  $\beta$ -blocking agents, a decrease in the pressure stretching afferent arterioles, and a reduction in the sodium levels perceived by cells of the distal tubules. The increase in plasma renin activity (PRA), if it persists for hours or days, can stimulate the synthesis and release of aldosterone by the adrenals; aldosterone, in turn, promotes the reabsorption of sodium by the distal tubules of the kidney and thus the retention of water by the vasculature. Thus, PRA and circulating aldosterone, in conjunction with vasopressin (antidiuretic hormone), are the humoral factors most instrumental in the regulation of fluids and electrolytes in mammalian systems.

It is thus not surprising that there is available an extensive literature on the effects of sedentary heat exposure and exercise in the heat and accompanying hypohydration on plasma levels of aldosterone (PA) as well as PRA. Kosunen et al. (26) and Adlerkreutz et al. (1) reported that just 20 min after sedentary exposure to the intense heat of a sauna (85-90°C), levels of both PA and PRA were significantly elevated even in experienced sauna users. Similarly, Dumoulin et al. (9) exposed test subjects to an 80°C ambient temperature for 20 min and observed increments in both PRA and PA. Even under more moderate environmental conditions, it has been well established that sedentary exposure to hot ambient conditions is accompanied by hormonal adaptations to reduce fluid and electrolyte loss; both PRA (11,12) and PA (12,14) play pivotal roles in these responses.

When physical exercise is superimposed upon the stress of a hot environment, then the endocrinological responses designed to protect and sustain fluid and electrolyte levels are exacerbated. Finberg et al. (13) reported that increments in PRA following exercise at 50°C could be lessened when the experiment was executed during the summer in comparison to the winter. When we dehydrated subjects by 5% of body weight, significant elevations were observed in PA and PRA even before exercise in the heat, and these increments persisted during a heat/exercise trial (16). More recently, we dehydrated test subjects by 3, 5, and 7% of original body weight, and reported that increasing the intensity of hypohydration from 3% to 5% was accompanied by increased PRA and PA during exercise in the heat (18). Interestingly, between 5% and 7% hypohydration, wherein no further absolute decrements in plasma volume were reported (31), we also observed no additional elevation in either PRA or PA (18). In these earlier studies drinking was permitted at the completion of each exercise interval only to the extent necessary to rehydrate each subject to the appropriate pre-exercise body weight.

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In the current studies we had the opportunity to assess the endocrinological responses of test subjects who underwent more moderate intensities of "voluntary dehydration" (22,24); in this case, the voluntary dehydration was the direct result of inconvenience in fluid ingestion. Test subjects were configured in chemical/biological protective garments, and in one experimental scenario were required to ingest fluids through a protective mask. These experimental contingencies elicited various levels of hypohy ration ranging between 0.84% and 4.04% of initial body weight. The numbers of test subjects and experimental trials allowed us to correlate endocrinological responses with hypohydration (% body weight loss) and other

experimental variables. Thus, this study was designed to assess the correlation between individual hypohydration levels and the intensity of the hormonal responses designed to offset decrements in fluid and electrolyte balance.

#### Materials and Methods

Sixteen (16) young adult male test subjects were recruited and thoroughly briefed on the nature and purpose of the current study; each signed an agreement of informed consent, and retained the right to withdraw at any time without retribution. Subjects were non-acclimated and were judged healthy by a physical examination. At 0645 h of each experimental day, subjects (Ss) reported to the climatic chamber facility for a light breakfast consisting of 450 ml of instant breakfast beverage, toast, butter, jam, and 450 ml of orange juice. Urinary specific gravity (refractometry) was tested postprandially to assure adequate hydration.

Immediately following this breakfast, a nude body weight was obtained and used to calculate percent body weight loss and sweat rates. Then Ss were instrumented (three point electrocardiographic monitoring, core temperature, and skin temperature of the forearm, calf, and chest) for both data collection and to assure safety criteria. All Ss wore socks and sneakers during all experimental trials. Following instrumentation test subjects were assigned to one of three clothing configurations on each experimental day - standard army fatigues, fatigues with chemical protective garments, and fatigues with chemical protective masks. These experimental configurations assured variable levels of thermal stress and difficulty in drinking since masked drinking required a tube which led from a canteen and connected to a mouthpiece of the mask. Additionally, when masks were worn,

two water-delivery systems were evaluated for efficacy in promoting rehydration: the current system (CS) and an innovative hand-pump delivery system (FF). Using the current system for masked drinking, Ss may raise the canteen above the level of the mask inlet and allow the fluid to flow by gravity and suction (current system, CS); the system under development (FIST-FLEXTM,FF, Wesleyan Co., Chicago, IL) provides a small hand-compression device near the collapsible canteen to serve as a hydraulic pump to deliver fluids through the mask. Each test subject participated in two trials only - one while masked and one in the non-masked configuration; at least 48h elapsed between randomized trials for each test subject. After test subjects were instrumented and appropriately garmented, careful assessment of clothed-instrumented body weight was made (Sauter balance, + 50 g accuracy).

Ss then entered a large environmental chamber which was maintained at moderate environmental conditions: T(dry bulb) = 31°C, T(wet bulb) = 19°C, relative humidity = 30%, and windspeed = 1.2 m/sec. These ambient conditions elicited a WBGT of approximately 23°C. Upon entry into the chamber, Ss were instructed to remain standing quietly for at least 20 min for equilibration of body fluids and fluid compartments (23). Following this interval, a small sample of blood was obtained by venepuncture from a superficial (antecubital) arm vein.

This was immediately followed by a 6 h interval of 50 min walk, 10 min rest with uninterrupted monitoring of core temperature, skin temperature, and heart rate. Subjects walked on large treadmills (4 man) set at a flat grade and at a rate of 1.11 m/s. Thus, if the entire test protocol were completed on a particular day, then Ss walked a total of 20 km. Water (31°C) was constantly available at arm's length from each volunteer; field water supplies were duplicated by adding 16 mg iodine/liter before distribution to canteens.

Ss were encouraged to drink <u>ad lib</u>, but information on hydration status (assessed during each rest period by weighing) was not provided. During each 10 min rest period, Ss sat quietly at their respective work station while physiological monitoring continued.

Upon completion of the final walk, Ss remained standing for acquisition of a final blood sample. Both blood samples were processed immediately. Hematocrit was immediately determined in triplicate by microcentrifugation while hemoglobin was quantitated using the cyanmethemoglobin method. Changes in plasma volume were calculated using the equations of Dill and Costill (8). Heparinized whole blood was centrifuged (4°C, 10000 g), and aliquots of plasma were frozen (-20°C) and stored for subsequent analysis. Angiotensin I levels were estimated from quantitation of plasma renin activity activity using radioimmunoassay test kits purchased from New England Nuclear Corp. (Billerica, MA) according to procedures noted in their technical bulletin. Wher angiotensin converting enzyme and angiotensinases are appropriately inhibited, it has been demonstrated that the accumulation of angiotensin I accurately reflects PRA. Ordinarily, control levels for healthy, normotensive upright men range from 1.0 to 4.0 ng angiotensin I formed per hour per ml plasma by this method (15). Plasma aldosterone levels (PA) were measured using cadioimmunoassay test kits obtained from Diagnostics Products Corp. (Los Angeles, CA) according to methods outlined in the respective technical bulletin. These methods and products ordinarily provide values ranging from 5-30 ng/dl for normotensive adult men. Plasma cortisol (PC) levels were estimated using radioimmunoassay test kits purchased from New England Nuclear Corp. (Billerica, MA) according to procedures outlined in their technical bulletin. Using these methods PC levels are generally reported to range from 5-25 ug/dl, with the variability dependent largely upon the time of day at which the blood samples are taken (27).

The effects of exercise in the warm environment were statistically analyzed by the paired t test for dependent data (28). Correlation cofficients were determined by linear regression analysis, and the null hypothesis was rejected at p<0.05.

#### Results

Fig. 1 demonstrates the effects of the several clothing configurations and exercise on circulating cortisol levels. PC was monitored in this experiment as a metric of generalized adrenocorticotrophic activity or stress level (17,20) induced by the combination of exercise, warm environment, and, in certain cases, encapsulation. The results indicate that for three of the experimental scenarios (fatigues, protective uniform, and protective uniform with mask, CS) there were no increases in PC levels. During a single experimental trial (protective uniform plus mask, FF) there did occur a significant (p<0.01) elevation in mean post-exercise cortisol level (22.1 ug/dl, post vs. 13.5 ug/dl, pre). The mean post-exercise value was somewhat skewed by a value of 44 ug/dl for a single test subject; interestingly, this particular individual also displayed one of the highest percentage body weight losses (3.8%) as well as PRA (18.8 ng/ml/h) and PA (76.2 ng/dl) subsequent to exercise while encapsulated.

Fig. 2 illustrates mean levels of PRA prior and subsequent to exercise in the various experimental configurations. The results clearly indicate that when clothed in standard Army fatigues and with ad lib drinking water available within reach, Ss completed the 6 h interval of 50/10 min work/rest cycles under these conditions with no effect on PRA. However, when the impermeable protective uniform and the protective uniform with the mask were added to the clothing configuration, the heat stress was increased considerably, and PRA was elevated significantly (p<0.025) in all three trials. It is interesting to observe that in the "fatigue trial" Ss lost 1.94% of their initial body weight while in the "fatigue plus protective uniform trial," the mean weight loss was 2.16% (p=ns). Moreover, when

individual weight losses, following exercise, were correlated with PRA (Fig.

3) the data manifested a highly significant (p<0.001) correlation. Depicted in this figure is the line of best fit for these data. Further, in the encapsulated and masked configurations, mean body weight losses were highest (2.56%, CS and 2.81%, FF) as were mean PRA (11.0 ng/ml/h FF, and 12.3 ng/ml/h, CS). In fatigues, post-exercise PRA = 2.78 ng/ml/h, and this increased to 8.27 ng/ml/h in the protective uniform trial.

Because of the correlation of PRA and percentage decrement in body weight we also examined the association between post-exercise PRA and rate of body weight loss (g/min), percentage change in plasma volume, and final (approximately maximal) heart rate. PRA was positively correlated with rate of weight (water) loss (r=0.47, t=2.82, P<0.01) and inversely with calculated decrement in plasma volume (r=0.51, t=-3.15, p<0.005). The data for final heart rate and PRA are depicted in Fig. 4, and demonstrate a close correlation between PRA and final heart rate (r=0.681, t=4.83, p<0.001).

Fig. 5 illustrates generally analogous results for the effects of the experimental configurations and exercise on PA. Thus, following the "fatigue trial", PA levels are not significantly different from those recorded prior to the experimental interval. However, in each of the three remaining configurations, post-exercise blood samples manifested significantly (p<0.05) increased levels of PA. Fig. 6 demonstrates that individual levels of PA are less closely correlated with percent body weight loss (p<0.01) than PRA (p<0.001, Fig. 3). Again, the line of best fit is shown for these data.

Discussion

Even at these relatively low levels of hypohydration (range = 0.84% -4.04%), our data show a strong correlation between PRA and PA responses to exercise in the various clothing configurations. Although there have been a considerable number of reports documenting the analogous responses of circulating levels of FRA and PA to sedentary heat exposure (3,14) and exercise in the heat (4,7), we have reported that the acquisition of heat acclimation has a greater moderating influence on PRA than PA responses (16). In a later experiment we reported (18) that hypohydration apparently had more notable effects on PRA than PA during exercise in the heat. These earlier results were confirmed by the current study wherein elevations in PRA were more closely correlated (r=0.635) with hyponydration as measured by absolute body weight loss than were increments in PA (r=0.469). While the responses of both PRA and PA to heat exposure and exercise in the heat are generally similar, Brandenberger et al. (5) used propranolol to increase PA responsiveness to heat exposure while PRA was significantly decreased. Likewise, Konikoff et al. (25) used salt loading to dissociate the effects of exercise in a hot, dry climate on PA and PRA responses. They reported (25) that whereas salt supplementation decreased PA during work in the heat, their regimen had no effect on PRA. Clearly, while plasma renin has been documented to partially control aldosterone secretion and release, experimental manipulation can dissociate these responses.

The work of Brandenberger et al. (4) indicated that whereas increments in PRA during exercise in the heat could be prevented by adequate water consumption, ingestion of isotonic fluid containing electrolytes and sucrose was necessary to offset completely increments in PA. Alternatively, Francis and MacGregor (19) reported that consumption of an electrolyte-rich fluid during exercise in the heat was equally effective in attenuating increments in

both PA and PRA. Geyssant et al. (21) assessed hormonal levels after a prolonged training period, and observed that while PA was unaffected, resting levels of PRA were reduced; they attributed this response to the increased plasma volume subsequent to training. Thus, there are indications that the responses of PRA to heat exposure/exercise in the heat may be more sensitive to the hydrational status of the test subjects while several studies have demonstrated that PA control may be affected more prominently by electrolyte status. This would be consonant with the results of the present study wherein PRA was more closely correlated with the intensity of hypohydration.

The design of the current study was ideal to elicit moderate, but variable, levels of hypohydration even at the mild WBGT (23°C) selected. Ordinarily, fluid intake during a march in a real or simulated tropic or desert environment is a function not only of work rate, clothing, and environmental conditions, but also of the temperature and palatability of the fluids available (24), time available for rehydration (29), accessibility and convenience of fluids (10) and a variety of factors such as thirst, gastric distention, and hot weather experience which may vary significantly among test subjects. While several of these variables were consistent among test subjects and test scenarios, the necessity to consume water through a tube/mouthpiece configuration in the masked ensemble assured variable levels of moderate hypohydration. Further, it is well-recognized that when individuals work acutely in warm or hot environments, fluid replacement will ordinarily not compensate for fluid losses in sweat and urine during the course of the exercise (2,6,22). This combination of factors provided the ideal range of graded hypohydration levels required to evaluate the association between PRA/PA and these levels.

We have concluded from these results that responses of fluid- and electrolyte-regulatory hormones to exercise even in a relatively moderate environment are markedly enhanced by protective garments which greatly exaggerate the level of heat stress. Further, decreasing the facility and convenience of rehydration by adding a through-mask/tube contingency for fluid consumption contributed to increased hypohydration, and hormonal levels were consistently highest during these trials. Even at the relatively moderate hypohydration levels elicited by these conditions, individual PRA and PA responses were significantly correlated to percent body weight loss.

Additionally, post-exercise PRA was positively correlated with the rate of weight (water) loss and inversely correlated with calculated changes in plasma volume, but were most closely correlated with final (maximal) heart rates. These data indicate that the intensity of endocrinological responses adaptive to fluid and electrolyte conservation are extremely sensitive to the level of hypohydration, and hence physiological cost, of the heat/exercise stress.

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The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense. Human subjects participated in these studies after giving their free and informed voluntary consent. Investigators adhered to AR 70-25 and USAMRDC Regulation 70-25 on Use of Volunteers in Research.

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### Figure Legend

Figure 1. Effects of the various clothing and accessory configurations on plasma cortisol prior and subsequent to exercise (1.11 m/s) in a moderate (23°C, WBGT) environment. Mean values ± SEM are depicted for each group: fatigues, n=7, protective uniform, n=7; protective uniform, mask-current system, n=8; protective uniform, mask-fist-flex, n=8. Each test subject performed two trials and the clothing configuration was randomized.

Figure 2. Effects of the various experimental configurations and exercise in a moderate environment on plasma renin activity. All conditions are as explained under Fig. 1.

Figure 3. Scatter plot and the line of identify of individual values of percentage of body weight loss during the exercise scenario and levels of plasma renin activity. The correlation coefficient was calculated by least squares regression analysis. Data for all subjects and trials are depicted.

Figure 4. Scatter plot and the line of identity of individual values of final heart rate (~ maximal heart rate) and levels of plasma renin activity. All conditions and parameters are as depicted under Figure 3.

Figure 5. Effects of the various experimental configurations and exercise in a moderate environment on levels of plasma aldosterone. All conditions are as explained under Figure 1.

Figure 6. Scatter plot of the correlation between percentage of body weight loss during the exercise scenario and aldosterone levels. All parameters are as indicated under Figure 3.

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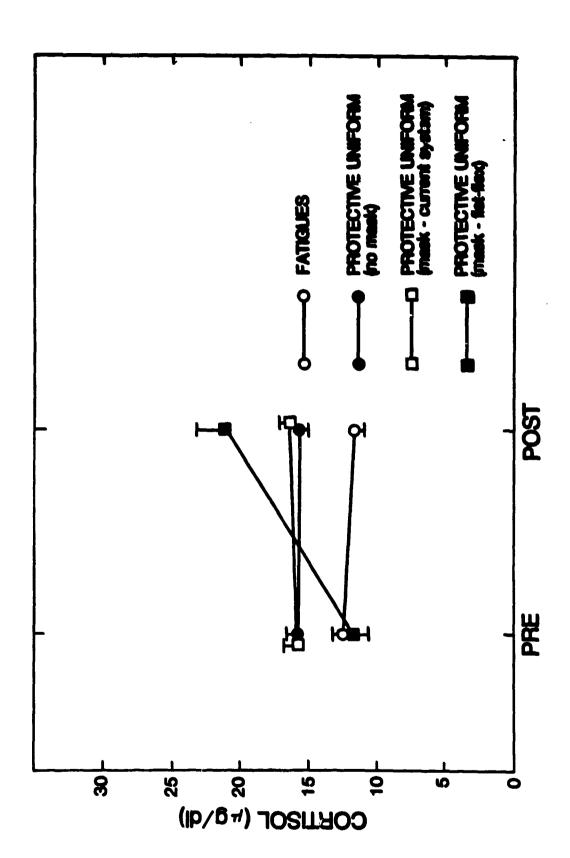
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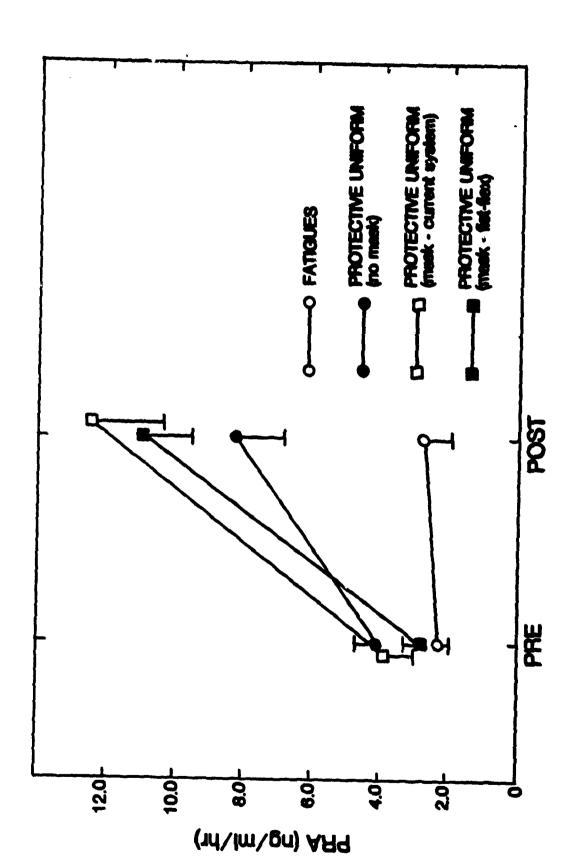
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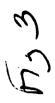
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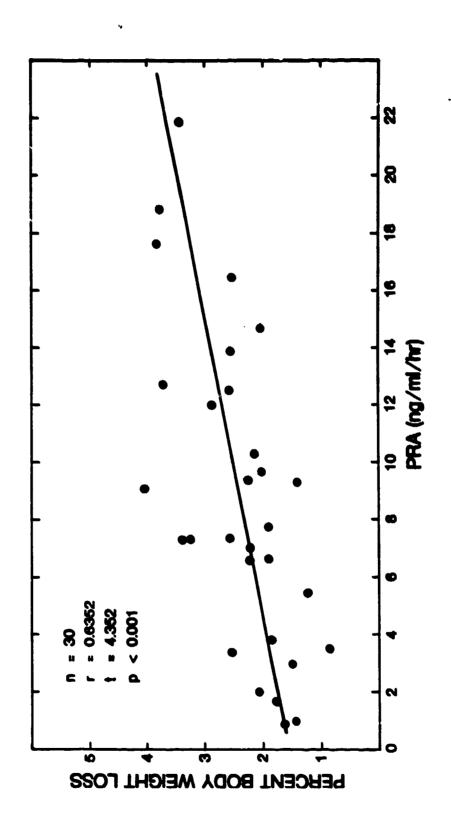
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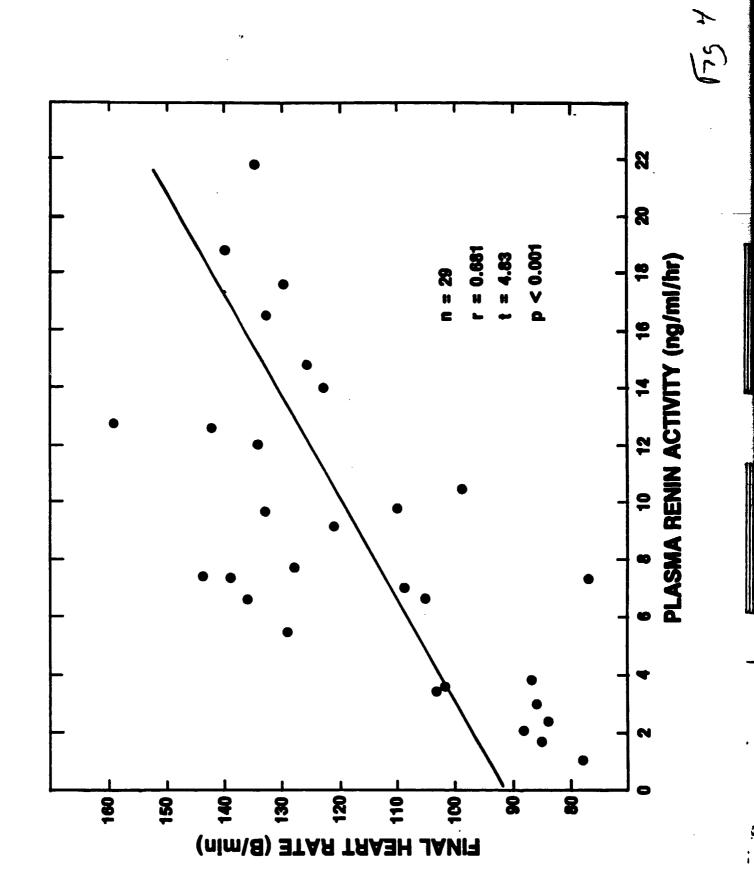


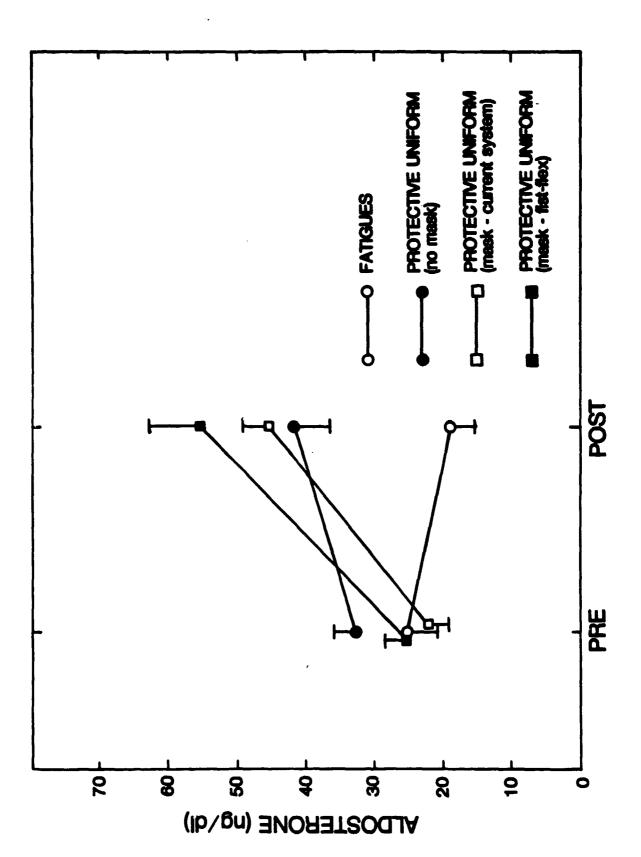


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Figs

